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This project evaluated the effect of housing 1 or 5 mice in identical sized cages on immunologic function. We have found that the C3H/HeJ male mouse, when placed from group housing to 1 animal housed per cage, has enhanced T cell related immune function in comparison to mice housed 5 per cage. Female C3H mice do not show this effect. The effect is present approximately 10 days after being placed into a single cage and lasts approximately 3-4 week. The altered immune reactivity is not related to corticosterone levels. Other cytokines, including IL-1 and macrophage colony stimulating factor are also produced in higher concentrations in individually housed mice. Bone marrow hematopoietic cell activity is greater in individually housed mice. Animals housed 1 per cage are significantly more resistant to infection with Candida albicans than animals housed 5 per cage. The time course of the development of enhanced resistance is similar to other immunologic parameters measured, appearing

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We have conducted a number of experiments which form a basis for understanding the immunologic alteration associated with housing conditions. Initially, we studied the C3H/HeJ inbred strain of male mice. We obtained the mice from a commercial supplier when they were approximately 6 weeks of age. They were subsequently placed into cages with either 5 or 1 animals per cage. The cages were 6 inches wide, 6 inches high and 12 inches long.

Effect of Housing on T and B Lymphocytes: Our first study involved inoculation of mice with sheep red blood cells. This antigen was selected because antibody production to sheep erythrocytes requires a T-helper lymphocyte to interact with the antibody producing B-lymphocyte before antibody to the sheep red blood cells will be produced. Thus, by using sheep red blood cells as an antigen, we were able to test the functional activity of T-helper lymphocytes and B-lymphocytes. Our data indicated that the number of B-lymphocytes producing antibody to sheep red blood cells was approximately 50% lower, when the C3H/HeJ male mice were housed 5 per cage, in comparison with those housed 1 per cage.

We then sought to determine whether the T-helper lymphocyte, the B-lymphocyte, or both lymphocytes were functionally altered by the housing conditions. We immunized mice with an antigen that does not require a T-helper lymphocyte to induce antibody production. The antigen which was used was polyvinylpyrrolidone (PVP), which directly activates B-lymphocytes. When the mice were inoculated with PVP, equal numbers of antibody-producing lymphocytes were obtained from animals housed either 1 per cage or 5 per cage. These results indicated that B-lymphocyte functional activity was not directly altered by the differential housing conditions. However, when a T-helper lymphocyte is required for the induction of antibody, less antibody occurs in the mice housed five per cage because of a possible altered activity of the T-helper lymphocyte.

To investigate further the function of T-helper lymphocytes, two additional experiments were conducted. The first involved stimulation of the T-lymphocytes with non-specific mitogenic agents. The rate of mitotic division induced in the T-lymphocytes was significantly greater in the lymphocytes from individually housed animals than the rate in group-housed animals. The second assay involved quantitation of the amount of interleukin-2 produced by the T-helper lymphocytes from group vs. individually housed animals. Significantly more interleukin-2 was produced by the T-helper lymphocytes from individually housed mice than the amount produced by the group-housed animals. The difference in interleukin-2 production could account for the lower amount of antibody production and the lesser response to mitogenic stimulation in the grouped mice. Finally, we determined whether differences occurred in the production of interleukin-1 between the group and individually housed mice. Using spleen lymphocytes, we found interleukin-1 production to be

the same in group and individually housed mice. This suggested that the only immunologic difference was at the level of the T-helper lymphocyte specifically involving interleukin-2 production. However, recently we studied IL-1 production by peritoneal-exudate macrophages from group and individually housed mice. The individually housed mice produced twice the amount of IL-1 as did the group-housed mice (Table 1). Thus, we now believe that macrophage/monocyte activity, as well as T-lymphocyte activity, are modified by housing conditions.

TABLE 1

Interleukin-1 Production by Peritoneal Exudate Cells of C3H/HeJ Male Mice Housed 1 or 5 Per Cage (Mean ± SE)
(Mouse Thymocyte Stimulation Assay)

	1/Cage	5/Cage
Supernatant	10,565 ± 2294 *	4,499 ± 744
PE Cell Lysate	35,660 ± 4480 **	22,975 ± 2772

* P <0.005 ** P < 0.02, in comparison to 5/cage

We have not yet determined why production of interleukin-2 or-1 differs in individually and group-housed male C3H/HeJ mice. These differences are not associated with the production of hormones by the adrenal gland. If the adrenal gland is removed from male C3H/HeJ mice and the animals are placed in the differential housing conditions, the immunologic differences between mice housed 5 or 1/cage persist. In addition, the concentration of corticosterone is the same in C3H/HeJ male mice, when housed 5 or 1 per cage. Thus, if one equates stress with a plasma elevation of corticosterone in the serum, the observation of an altered immune function with differential housing would not be considered to be due to stress. However, other hormones or nervous system pathways may become altered based on housing. As we better understand these pathways, we may have to redefine the biochemical changes which are considered to be synonymous with stress.

Another series of experiments involved the study of female C3H/HeJ mice. When the female animals were housed 5 or 1 per cage, differences were not detected in any of the measured immunologic parameters between the 2 groups. Thus, an interaction exists between sex of the animal and the housing conditions in C3H/HeJ mice. However, it is important to caution against generalization. The CD-1 strain shows a similar increase of immunologic reactivity in individually housed male and female animals. Thus, CD-1 females differ from the C3H/HeJ females.

Effect of Housing on Resistance to Infection: In addition to the in vitro assays, in vivo studies of resistance to infection with Candida albicans were also performed. Individually or group-housed animals were inoculated intravenously with Candida albicans and the number of organisms which had to be injected to infect 50% of the animals' kidneys was determined. Individually

housed animals were significantly more resistant to infection, requiring approximately 2.5 times more organisms to infect 50% of the kidneys of male C3H/H3J mice.

Phagocytosis: The capacity of peritoneal macrophages from mice caged 1/cage vs. 5/cage to carry on phagocytosis was determined by direct counts of ingested heat-killed cells of C. albicans. In six experiments, peritoneal macrophages from mice caged 1/cage were consistently 1.5 times more active than macrophages from mice caged 5/cage (Table 2) in ingesting the dead cells of C. albicans. In all cases, the mice were housed for ten days either 1/cage or 5/cage before sacrifice and recovery of the peritoneal macrophages.

TABLE 2

Comparison of Phagocytic Activities of Macrophages From Mice Housed 1/Cage vs. 5/Cage. Percentage of Peritoneal Macrophages That Have Ingested at Least One C. albicans Cell

	1/Cage	5/Cage
Average	31.5*	21.4
S.E.	3.5	4.6

*Average reading of counts from a minimum of 200 peritoneal macrophages in each of six separate experiments.

Response of Bone Marrow Cells to Colony Stimulating Factor (CSF): Marrow cells from the tibias and fibulas of male C3H/HeJ mice housed either 1/cage or 5/cage were incubated in McCoy 5a agar medium containing CSF. On the seventh day of incubation, approximately 1.6 times more colonies, each containing 50-100 or more cells, could be counted in the plates with marrow cells from mice housed 1/cage than in the plates with cells from mice housed 5/cage (Table 3). Thus, mice housed 1/cage have marrow cells more responsive to CSF in the capacity of the hematopoietic cells to form colonies.

TABLE 3

Comparison of Numbers of Colonies Developing From Marrow Cells from C3H/HeJ Male Mice Housed 1/Cage or 5/Cage in McCoy 5a Medium Containing Colony Stimulating Factor

Note:Colonies were counted in each of three plates from each group of mice (1/cage vs. 5/cage in three separate experiments)

	1/Cage	5/Cage	Average	Ratio
#1	75,58,49	40,43,26	60.7 vs. 36.3	1.67
#2	22,40,7	13,10,23	23.0 vs. 15.3	1.50
#3	8,16,10,24	8,13,7	14.5 vs. 9.3	1.56

Experiments were then initiated to compare the capacities of

spleens from mice caged 1/cage vs. 5/cage to produce CSF. Spleen cells were incubated at a concentration of 4 x 10^6 cells/ml in RPMI 1640 supplemented with 5% human serum, 5% of a 1:15 dilution of reconstituted pokeweed mitogen (PWM) plus penicillin (20 u/ml) and streptomycin (20 ug/ml). Five ml were incubated for 4 days in 25 cm Falcon plastic tissue culture flasks at 37° C in 5% CO_2. The medium was decanted and centrifuged at 1000 RPM for 10 minutes. The supernatant was then filtered through a 0.22 um Millipore filter. The quantity of CSF present was determined as above.

In two separate experiments, spleen cells from mice housed 1/cage released about twice the amount of CSF as spleen cells from mice housed 5/cage. Thus, male C3H/HeJ mice housed 1/cage are more active than mice housed 5/cage in the capacities to respond to and to release colony stimulating factor.

TABLE 4

Capacity of spleen cells from C3H/HeJ male mice housed 1/cage vs. 5/cage to produce colony stimulating factor

	1/Cage	5/Cage	
Exp. 1*	72,60	25,24	
Exp. 2	68,65,42,42	33,36	
Average	60.1	29.5	Ratio=2.04

* Counts of colonies from normal bone marrow cells exposed to spleen extract from mice housed 1/cage or 5/cage

Differential Housing Produces a Transient Alteration of Immune Function: By three weeks after being placed into the differential housing conditions, the immune reactivations as measured by (a) non-specific mitogen reactions, and (b) number of spleen lymphocytes producing antibody to sheep erythrocytes and (c) resistance to C. albicans were similar, regardless of the number of animals housed per cage. Thus, the immunologic difference is a transient difference and seems to be related not only to the number of mice housed/cage, but also to a change in the environment.

Genetics and Corticosterone: The immune system is regulated by a series of genes in the major histocompatibility complex (MHC). We wanted to determine whether the MHC was involved in the altered immune function with differential housing. Within the MHC, there are two genetic loci of primary immunologic importance in the mouse, i.e. H2D and H2K. Strains of animals were selected for study which shared neither the H2D and H2K loci with the C3H/HeJ animals or which shared either or both of these loci with C3H/HeJ. One strain, C3H.SW/SNJ (which shares neither the H2D or H2K loci with C3H/HeJ) had significantly more lymphocyte-producing antibody to sheep erythrocytes in the spleens of the individually housed mice,in comparison with the

group-housed mice. Also, the C3H.SW/SNJ male mice did not have a difference in their corticosterone levels based on housing. Thus, in this regard they were identical to the C3H/HeJ mice. This also indicates that corticosterone levels are not primarily involved in the differential immune response and that the major histocompatibility locus is not involved in the immunologic differences based on housing.

The preliminary data indicate that a variety of immunologic activities are altered by housing conditions. It is likely that the ability to resist infectious disease can be altered by the immune changes. Thus, it is important that these changes be more completely characterized and the mechanism of the alterations be determined. Once the mechanisms are understood, further studies can be proposed to characterize the mechanism of a possible environmental influence on susceptibility to infection and for activation of latent infection.

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